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The Genetic Basis of Specificity in Dinoflagellate-Invertebrate Symbiosis

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The prime goals of this award were: (1) to study the compositon of the cell walls of symbiotic dinoflagellates, (2) analyse the chemical composition of the glycoproteins exuded by symbiotic dinoflagellates and (3) sequence the SSU rRNA gene from the symbiotic dinoflagellate *Symbiodinium pilosum* and assess its phylogenetic position relative to other protists. All three of these goals have been accomplished, and manuscripts or published papers describing the observations are attached. Briefly, the cell walls of symbiotic dino-flagellates are composed of cellulose and proteins, but whether any of the proteins are involved in recognition of symbionts by hosts remains unresolved. The large molecular weight glycoproteins exuded by symbiotic dinoflagellates have been characterized, and polyclonal antibodies have been prepared against them. We have preliminary evidence that show that the glycoproteins exuded by the symbionts in culture are also exuded *in hospite*. Analyses of the SSU rRNA sequence of *S. pilosum* shows that it is clustered among the dinoflagellates which appear to share a common ancestry with the Apicomplexa and the Ciliata.

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The genetic basis of specificity in dinoflagellate-invertebrate symbiosis. (Supplement - Nucleotide sequence of the SSU rRNA gene of *Symbiodinium pilosum*)

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OBJECTIVE: The prime goals of this award were: (1) to study the compositon of the cell walls of symbiotic dinoflagellates, (2) analyse the chemical composition of the glycoproteins exuded by symbiotic dinoflagellates and (3) sequence the SSU rRNA gene from the symbiotic dinoflagellate *Symbiodinium pilosum* and assess its phylogenetic position relative to other protists.

ACCOMPLISHMENTS: In the study of the mechanisms that are involved in producing the observed specificity in the symbioses between dinoflagellates and marine invertebrates, there are several hypotheses that can be tested. One hypothesis is that specific algae possess a suite of specific molecules on their surfaces (cell walls) that interact with animal cell plasmalemma during the phase of initial contact. Such specific ligand-receptor interactions could be responsible for the phagocytosis of the algae by the animal cells, the "inhibition" of phago-lysosome fusion, and the ultimate sequestration of the algae at the base of hosts' endodermal cells. In this case, the associants would recognize each other.

An alternate hypothesis is that a specific alga produces some component that is sufficiently similar to, for example, a product of the host's histocompatibility genes. On contact, the host would not recognize the alga as a foreign entitity.

Distinguishing between these two hypothesis is currently not possible. To test whether the algal cells do possess potential molecules that could be involved in recognition e.g. glycoproteins, we isolated cell walls, solubilized them and analyzed the components by SDS-PAGE. We found that the

cell walls of the four species of symbiotic dinoflagellates studied contained cellulose and associated polypeptides of molecular sizes ranging from 14 - about 200kDa.. This observation represents the first demonstration of polypeptides associated with dinoflagelate cell walls. A potentially more significant observation that was made during this same study was the discovery that symbiotic dinoflagellates release *in vitro*, water-soluble large molecular weight glycoproteins. This observation raises the possibility that components of these exuded glycoproteins could be the "signals" passing between symbiont and host. It is obviously important to this concept to demonstrate that the release of glycoproteins also occurs *in hospite*. To this end, we prepared polyclonal antibodies against the exudate from *Symbiodinium microadriaticum*, the specific symbiont of the jellyfish *Cassiopeia xamachana*, and, using immunolocalization techniques at the level of the electron microscope, could detect evidence of algal glycoproteins in host tissues in the resynthesized symbiosis. Control apo-symbiotic animals demonstrated no actvity, nor was any activity observed using pre-immune serum. These observations are consistent with the interpretation that (a) glycoproteins are released by the algae *in hospite* and (b) that all these glycoproteins are not digested and hence are not serving a primary nutritive function. The additional observation that host nuclei in cells in a specific region of the scyphistomae showed evidence of reacting with the exudate could indicate exudate-host DNA binding, and could be the basis for the initiation of the morphogenetic sequence of events leading to strobilation. We are currently involved in gel retardation analyses using aposymbiotic host DNA and the exudate to determine protein-DNA binding.

Analyses of the exuded glycoproteins by HPLC shows that they are carbohydrate rich, containing glucose, galactose, fucose, galactosamine, glucoseamine and uronic acids. No evidence of either mannose or scialic acid was observed. The protein component of the exuded glycoproteins is rich in serine, glycine, and alanine, and contains significant levels of threonine proline, valine, lysine, leucine and isoleucine. This represents a much wider suite of amino acide that those represented in the small molecular weight soluble "translocated" pool. A paper reporting the sugar and amino acid composition, and the lectin-binding characteristics of the exuded glycoproteins is currently in preparation.

The complete sequence of the SSU rDNA from *Symbiodinium pilosum* has been determined, and phylogenetic reconstruction analyses by both cladistic and phenetic methods show that the dinoflagellates are more closely affiliated with the Apicomplexa than with the Ciliata. Among the dinoflagellates, *Crypthecodinium cohnii* is more closely linked to the apicomplexans than either *S. pilosum* or *Prorocentrum micans*. This latter observation brings into doubt the ancestral position traditionally ascribed to the prorocentroid dinoflagellates.

List of papers (published, in press, and submitted) during the period of support.

Govind, N.S., Roman, S.J., Iglesias-Prieto, R., Trench, R.K., Triplett, E.L., and Prézelin, B.B. 1990. An analysis of the light-harvesting peridinin-chlorophyll *a* -proteins from dinoflagellates by immunoblotting techniques. *Proc. R. Soc. Lond.* B **240**, 187 - 195 (5 copies submitted previously).

Matta, J.L. and Trench, R.K. 1991.The enzymatic response of the symbiotic dinoflagellate *Symbiodinium microadriaticum* (Freudenthal) to growth *in vitro* under varied oxygen tensions. *Symbiosis* **11**, 31 - 45.(5 copies enclosed)

Markell, D.A., Trench, R.K., and Iglesas-Prieto, R. 1991. Macromolecules associated with the cell walls of symbiotic dinoflagellates. *Symbiosis* **12**, (one copy of galley enclosed; 5 copies of reprints will be forwarded upon receipt).

Trench, R.K. 1991. Current trends in the study of microalgal-invertebrate symbiosis. In: *Encyclopedia of Microbiology*. J. Lederberg (ed). Academic Press. (in press;one copy of manuscript enclosed; 5 copies of reprints will be forwarded upon receipt).

Sadler, L.A., McNally, K.L., Govind, N.S., Brunk, C.F., and Trench, R.K. 1991. The nucleotide sequence of the small subunit ribosomal RNA gene from *Symbiodinium pilosum*, a symbiotic dinoflagellate. *Current Genetics*.(submitted, manuscript enclosed).

Matta, J.L., Govind, N.S., and Trench, R.K. 1991. Polyclonal antibodies against iron-superoxide dismutases from *E. coli* B cross-react with superoxide dismutases from the symbiotic dinoflagellate *Symbiodinium microadriaticum*. *Nature* (submitted, manuscript enclosed).

Iglesias-Prieto, R., Govind, N.S. and Trench, R.K. 1991. Apoprotein composition and spectro scopic characterization of the water-soluble peridinin-chlorophyll *a*-proteins from three symbiotic dinoflagellates. *Proc. R. Soc. Lond.* B.(submitted, manuscript enclosed).